PB84-246230

Relationship between the Metabolism of Haloacetonitriles and Chloroform and Their Carcinogenic Activity

(U.S.) Health Effects Research Lab.
Cincinnati, OH

Sep 84

U.S. Department of Commerce
National Technical Information Service
NTIS

PB84-246230

EPA-600/D 84-203
September 1984

"RELATIONSHIP BETWEEN THE METABOLISM OF HALOACETONITRILES AND CHLOROFORM AND THEIR CARCINOGENIC ACTIVITY"

Michael A. Pereira, F. Bernard Daniel[1] and Edith L. C. Lin

Health Effects Research Laboratory
U.S. Environmental Protection Agency
26 W. St. Clair Street
Cincinanti, Ohio 45268

[1]Present address: F. Bernard Daniel
National Institute for Occupational Safety and Health
Experimental Toxicology Branch
4676 Columbia Parkway
Cincinnati, Ohio 45226

Corresponding Authors: Dr. Michael A. Pereira
U.S. Enviornmental Protection Agency
Health Effects Research Laboratory
26 West St. Clair Street
Cincinnati, OH 47568

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/D-84-203		PB84 246230

4. TITLE AND SUBTITLE	5. REPORT DATE
Relationship Between the Metabolism of Haloacetonitriles and Chloroform and Their Carcinogenic Activity	September 1984
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Michael A. Pereira, F. Bernard Daniel, and Edith L. C. Lin	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS TMD/HERL/ORD/USEPA 26 West St. Clair Street Cincinnati, OH 45268	10. PROGRAM ELEMENT NO. ABMC1A 11. CONTRACT/GRANT NO. In-House
12. SPONSORING AGENCY NAME AND ADDRESS HERL/ORD/USEPA Research Triangle Park, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED Conference Proceedings 14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES P.O. Michael A. Pereira

16. ABSTRACT

Halogenated acetonitriles, found in chlorinated drinking water, are formed by the reaction of chlorine with algae, fulvic acid, and proteinaceous material in the source water. The metabolism of halogenated acetonitriles was investigated in relation to their possible carcinogenic activity. Chloroacetonitrile (CAN), dibromoacetonitrile (DBAN), dichloroacetonitrile (DCAN), bromochloroacetonitrile (BCAN), and trichloroacetonitrile (TCAN) were shown to bind the nucleophilic trapping agent, *p*-nitrobenzylpyridine, without metabolic activation and to produce strand breaks in the DNA of cultured human CCRF-CEM cells. In addition, DCAN bound DNA in vitro without metabolic activation producing an adduct to adenine, and DBAN bound rat-liver DNA following oral administration.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to public	unclassified	19
	20. SECURITY CLASS (This page) unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

INTRODUCTION

The chlorination of water for the purpose of drinking results in the formation of low molecular weight by-products including halogenated acetonitriles and trihalomethanes. Dihaloacetonitriles have been found in chlorinated drinking water at a concentration of 0.3-8.1 ppb (1,2). Haloacetonitriles are direct-acting alkylating agents that bind the dye, p-nitrobenzylpyridine (3), and are direct-acting mutagens in Salmonella typhimurium (4). Several haloacetonitriles induced tumors when applied to the skin of SENCAR mice (4). Thus, haloacetonitriles would appear to possess carcinogenic activity. Chloroform, a trihalomethane found in chlorinated drinking water (5-7), has been shown, in a long-term bioassay, to cause liver tumors in B6C3F1 mice and kidney tumors in Obsorne-Mendel rats (8). In this communication, we summarize our results on the possible mechanisms of the apparent carcinogenicity of haloacetonitriles and chloroform especially as related to their metabolism.

HALOACETONITRILES

DNA Alteration/Damage

The initiating event of chemical carcinogenesis is believed to be the interaction of the carcinogen with DNA. We determined the ability of the haloacetonitriles in the absence of a metabolic activation system to bind to various polynucleotides. Dichloroacetonitrile (DCAN)-^{14}C bound polyadenylic acid in the absence of a metabolic activation system to produce an adduct that after acid hydrolysis, was isolated by HPLC (9). No adducts were eluted from the HPLC column when an hydrolysate of polyguanylic acid previously incubated with DCAN-^{14}C was analyzed. When calf thymus DNA was incubated with DCAN-^{14}C and the resultant product hydrolyzed, the HPLC elution profile contained a peak of radioactivity identical to that obtained with polyadenosine (Figure 1).

We then determined the ability of dibromoacetonitrile (DBAN) to covalently bind to liver and kidney DNA in rats. When administered orally to rats, DBAN-^{14}C appeared to bind covalently to liver and kidney DNA (Table I). However, we are still in the process of attempting to demonstrate that the radioactivity present in the isolated DNA results from actual DNA adducts and not from contaminating protein adducts or from de novo DNA synthesis. The covalent binding of haloacetonitriles to DNA would indicate potential carcinogenic activity.

The ability of a carcinogen to interact and to alter DNA can be demonstrated by an induction of DNA strand breaks. Haloacetonitriles induced DNA strand breakage in cultured human CCRF-CEM cells (Table II). Trichloroacetonitrile (TCAN) was a very potent DNA strand breaker while DCAN and chloroacetontrile (CAN) were marginally active. DBAN and bromochloroacetonitrile (BCAN) were of intermediate activity. Therefore, the interaction of haloacetonitriles with DNA results in alterations that can cause DNA strand breakage.

Metabolism

We have proposed a scheme for the metabolism of haloacetonitriles that is presented in Figure 2. Haloacetonitriles can be metabolized with the release of cyanide by two different mechanisms: 1) non-enzymatic hydrolysis and 2) oxidation via the mixed function oxidase (MFO) of the endoplasmic reticulum. According to this metabolic scheme CAN will yield formaldehyde by non-enzymatic hydrolysis and formylcyanide and formyl chloride by MFO metabolism; DCAN will yield formyl cyanide and formyl chloride by non-enzymatic hydrolysis and phosgene and cyanoformyl chloride by MFO metabolism; and TCAN will yield phosgene and cyanoformyl chloride by non-enzymatic hydrolysis. That these reactions occur in vivo was evident by the excretion of thiocyanates in

the urine of rats administered the haloacetonitriles (Table III). Thiocyanate is produced by the further metabolism of the released cyanide by rhodanese (EC 2.8.1.1). The extent of urinary excretion in 24 hours of thiocyanates as the percentage of the administered dose was greater for CAN and BCAN than for DBAN and DCAN, which, in turn, were much greater than for TCAN. Thus, the urinary excretion of thiocyanates would indicate that the haloacetonitriles are absorbed systemically. In conclusion, the haloacetonitriles appear capable, after systemic absorption, of binding and/or damaging DNA. Since DNA alteration/damage is believed to be an important initiating event in chemical carcinogenesis, the haloacetonitriles warrant further study in order to determine whether their oral administration can cause cancer or some other disease having a genotoxic basis (mutation).

CHLOROFORM

In a U.S. National Cancer Institute sponsored study, chloroform administered by stomach gavage in corn oil was demonstrated to induce liver tumors in mice (8). However, the lack of genotoxic activity of chloroform has resulted in the hypothesis that the mechanism of chloroform hepatocarcinogenicity is tumor promotion (12,13). As result of these observations, we tested chloroform for tumor promoting activity in mouse liver. In summary, our study consisted of administering to 15 day old outbred Swiss mice either 0, 5 or 20 mg/kg ethylnitrosourea (ENU) dissolved in saline. At weanling, some of the mice from each ENU treatment group started to receive 1800 ppm chloroform or 500 ppm sodium phenobarbital (positive control for mouse liver tumor promotion) in their drinking water. The animals were sacrificed at 51 weeks of age (after 46 weeks of chloroform exposure) and complete histopathological evaluation performed on the liver, lung, kidneys and all gross lesions. ENU at 5 and 20

mg/kg, induced a dose dependent increase in liver adenomas and in hepatocellular carcinomas (Table IV). The subsequent administration of chloroform inhibited the occurrence of liver tumors both in animals that did not received ENU and in animals that received ENU. Therefore, in Swiss mice, chloroform administered in the drinking water acts as an inhibitor of hepatocarcinogenesis.

The inhibitory action on hepatocarcinogenesis of chloroform in our study is in contrast to the National Cancer Institute sponsored study where chloroform demonstrated hepatocarcinogenicity (8). In the National Cancer Institute sponsored study the chloroform was administered to B6C3F1 mice in corn oil by stomach gavage (8). In our study the strain of mice was CD-1 (Swiss) annd the route of administration was drinking water. Preliminary results (gross observation) of an ongoing study in B6C3F1 indicates that chloroform administered in the drinking water to this strain of mice also inhibits both "spontaneous" and chemically (ENU and diethylnitrosamine)-induced hepatocarcinogenesis. The 1800 ppm dose of chloroform administered in the drinking water is very similar to the amount administered by stomach gavage in the long term bioassay. However, the pharmacokinetic consequences of a bolus vs. continuous dose might be the reason for these differing effects of chloroform.

An alternative possibility for the differing results in the two studies is that there is a synergistic interation between the chloroform and the corn oil resulting in the hepatocarcinogenic activity. In fact, increasing fat consumption, especially polyunsaturated fat, of mice has been shown to result in an increased incidence of "spontaneous" liver tumors (14-16). In rats, the concurrent feeding of a lipotrope-deficient, high polyunsaturated fat diet with diverse carcinogens such as aflatoxin B_1, dibutylnitrosamine, diethylnitrosamine or N-2-fluorenylacetamide enhanced their hepatocarcinogenicity (17,18).

The mechanism of the enhancement by polyunsaturated fat might result 1) from the induction of liver mixed function oxidase activity by polyunsaturated fat as compared to saturated fat (19,20) or 2) from the hepatic toxicity of a lipotrope-deficient, high polyunsaturated fat diet. These metabolic alterations in the liver produced by high levels of polyunsaturated fat might change the hepatic activity of chloroform from an inhibitor (administered in drinking water), to an inducer (administered in corn oil) of hepatocarcinogenesis. Further studies, especially in the area of metabolism are required in order to determine the mechanisms by which chloroform can under different circumstances act either as an inhibitor or as an inducer of liver cancer.

The research described in this article has been reviewed by the Health Effects Research Laboratory and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Table I. Binding of Dibromoacetonitrile to Liver and Kidney DNA in Rats[a]

Organ Time (hrs)	DNA Binding μmole/mg DNA
a. Liver	
4	43.8 ± 12.9[b]
24	434 ± 153
b. Kidney	
4	10.8 ± 1.0
24	62.1

[a]The rats were administered 0.52 mmole/kg DBAN-^{14}C (8.6mCi/ mmole) and were sacrificed either 4 or 24 hrs later. The DNA ws isolated by phenol extraction and the amount of radioactivity and DNA measured as previously described (10).

[b]Results are means ± S.E. for 2-4 rats each except for the 24 hr kidney in which the kidneys from 2 rats were pooled.

Table II. DNA Strand Breaks Produced by 1 hr Exposure to Haloacetonitriles in Human CCRF-CEM cells[a]

Haloacetonitrile	DNA Strand Break Breaks/Cell/μ M ± S.D.
TCAN	74.5 ± 27.6[c]
BCAN	12.8 ± 3.7[c]
DBAN	6.9 ± 0.4[b]
DCAN	4.3 ± 1.1[b]
CAN	2.0 ± 1.4[b]
Controls	
DMS[d]	158 ± 16[c]
MMS[d]	33 ± 2.5[c]

[a]The human CCRF-CEM cells were incubated in serum free medium with different dose levels of the haloacetonitriles for 1 hr. The number of breaks/cells were determined by the previously described alkaline unwinding procedure using hydroxylapatite to separate single and double stranded DNA (11). The number of breaks/cell/μM was calculated from the slope of the dose-response curve.

[b]Significantly greater than solvent control ($P < 0.01$) by the Student t test.

[c]Significantly greater than solvent control ($P < 0.01$) by the Student t test.

[d]DMS, dimethysulfate; MMS, methylmethane sulfonate.

Table III. Urinary Excretion of Haloacetonitriles as Thiocyanates[a]

Haloacetonitriles	Urinary Thiocyanates % of dose
CAN	14.2 ± 3.8[b]
BCAN	12.8 ± 1.5
DCAN	9.28 ± 1.09
DBAN	7.67 ± 2.13
TCAN	2.25 ± 0.82

[a]The haloacetonitriles (0.75 mmole/kg) were administered by gavage in tricaprylin to male rats and a 24 hr. urine sample obtained (3). For each individual animal, the thiocyanates content in the urine collected during the 24 hr. prior to the administration of the haloacetonitriles was substrated from the value obtained for the 24 hr. after the administration of the haloacetonitrile.

[b]Results are mean ± S.E. for 5-6 rats.

Table IV. Effect of Subsequent Treatment with Chloroform on the Incidence of Liver Tumors in Male Mice Initiated by Ethylnitrosourea at 15 Days of Age

Group	Treatment	N[b]	No. Animals with Adenomas	Adenomas/ Animal	No. Animals with Carcinomas	Carcinomas/ Animal
	Ethylnitrosourea (ENU) mg/kg					
1	20	30	22(73)[c]	3.1	10(33)	0.83
2	5	39	8(21)	0.51	2(5)	0.10
3	0	37	2(5)	0.19	2(5)	0.08
	ENU + 1800 ppm Chloroform					
4	20	29	12(41)	1.0	5(17)	0.21
5	5	25	1(5)	0.05	0(0)	0
6	0	23	0(0)	0	0(0)	0
	ENU + 500 ppm Phenobarbital					
7	20	25	22(88)	2.2	17(68)	2.5
8	5	36	14(39)	0.56	10(28)	0.44
9	0	30	6(20)	0.27	6(20)	0.23

[a]Fifteen day old mice were administered by stomach gavage the ENU and at weanling the animals started to receive either 1800 ppm chloroform or 500 ppm Phenobarbital (positive control for liver tumor promoter). The animals were sacrificed at 51 weeks of age.

[b]N is the number of animals.

[c]Numbers in parentheses are the percentage of animals with the lesion.

FIGURES

Figure 1. Elution profile of the products of DCAN binding to DNA. DCAN-^{14}C was incubated with calf thymus DNA at 37°C and for 22 hrs. After isolation of the DNA and extraction of unbound DCAN, the DNA was hydrolyzed by treatment with 0.1N HCl at 70°C for 30 mins. The hydrolysate was analyzed by HPLC using a Whattman Magnum 9 SCX column eluted with 0.025M ammonium phosphate, pH 4.0 for 10 mins followed by a linear gradient to 0.2M ammonium phosphate, pH 4.0 in 10% methanol for 15 mins. at a flow rate of 1.0 ml/min. The adenine (Ade) and guanine (Gua) in the hydrolyzate were followed by absorbance at 260 nm.

Figure 2. Proposed methabolic pathways for the metabolism of chloroacetonitrile, dichloroacetonitrile and trichloroacetonitrile.

REFERENCES

1. Oliver, B. G. "Dihaloacetonitriles in Drinking Water: Algae and Fulvic Acid as Precursors", Envir. Sci. Technol. 17:80-83 (1983).
2. Trehy, M. L. and T. I. Bieber. "Detection, Identification and Quantitative Analysis of Dihaloacetonitriles in Chlorinated Natural Waters," in Advances in Identification and Analysis of Organic Pollutants in Water, Vol. 2, L. H. Keith, Ed. (Ann Arbor, MI: Ann Arbor Science Publishers, Inc., 1981), pp 941-975.
3. Pereira, M. A., L.-H. C. Lin and J. K. Mattox. "Haloacetonitriles Excretion as Thiocyanate and Inhibition of Dimethylnitrosamine Demethylase: A Proposed Metabolic Scheme", J. Toxicol. Environ. Health (1984). In press.
4. Bull, R. J. and M. Robinson. "Carcinogenic Activity of Haloacetonitriles and Haloacetone Derivatives in the Mouse Skin and Lung:, in Fifth Conference on Water Chlorination: Environmental Impact and Health Effects, R. L. Jolly, R. Bull, R. Cumming, W. Davis, S. Katz and M. Roberts, Eds. (Ann Arbor, MI: Ann Arbor Science Publishers, Inc., 1984). In this volume.
5. Rook, J. J. "Formation of Haloforms during Chlorination of Natural Waters," Water Treatment Exam., 23:234-243 (1974).
6. Bellar, T. A., J. J. Lichtenberg, and R. C. Kroner. "The Occurrence of Organohalides in Chlorinated Drinking Water", J. Am. Water Works Assoc. 66:703-706 (1974).
7. Symons, J. M., T. A. Bellar, J. K. Carswell, J. Demarco, K. L. Kropp, G. G. Robeck, D. R. Seeger, C. J. Slocum, B. L. Smith, and A. A. Stevens. National Organics Reconnaissance survey for Halogenated Organics" J. Am. Water Works Assoc. 67:634-647 (1975).

8. "National Cancer Institute, Carcinogenesis Bioassay of Chloroform" Natl. Tech. In form. Service No. PB 264018/AS (Washington, D.C. Natl. Cancer Inst., 1976).

9. Daniel, F. B., M. A. Pereira, L.-H. C. Lin, D. L. Haas, J. K. Mattox, and K. Schenck. "Genotoxicity of Haloacetonitriles," Toxicologist 3:37 (1983).

10. Pereira, M. A., L. H. C. Lin and L. W. Chang. "Dose-Dependency of 2-Acetylaminofluorene Binding to Liver DNA and Hemoglobin in Mice and Rats," Toxicol. Appl. Pharmacol. 60:472-478 (1981).

11. Daniel, F. B., D. H. Haas and S. M. Pyle. "Quantitation of Chemically Induced DNA Strand Breaks in Human Cells Via an Alkaline Unwinding Assay," Chem. Biol. Interact. Submitted for Publication.

12. Pereira, M. A., L.-H. C. Lin, H. M. Lippitt, and S. L. Herren. "Trihalo methanes as Initiators and Promoters of Carcinogenesis", Environ. Hlth. Persp. 46:151-156 (1982).

13. Reitz, R. H., T. R. Fox, and J. F. Quast. "Mechanistic Considerations for Carcinogenic Risk Estimation: Chloroform," Environ. Hlth. Persp. 46:163-168 (1982).

14. Tannenbaum, A. "The Genesis and Growth of Tumors. III Effects of a High-Fat Diet," Cancer Res. 2:468-475 (1942).

15. Silverstone, H. and A. Tannenbaum. "The Influent of Dietary Fat and Riboflavin on the Formation of Spontaneous Hepatomas in the Mouse," Cancer Res., 11:200-206 (1951).

16. Gellatly, J. B. M. "The Natural History of Hepatic Parenchymal Nodule Formation in a Colony of C57BL Mice with Reference to the Effect of Diet," in Mouse Hepatic Neophasia, W. H. Butler and P. M. Newberne, Eds. (New York: Elsevier Press, 1975) pp 77-110.

17. Rogers, A. E. "Influence of Dietary Content of Lipids and Lipotropic Nutrients on Chemical Carcinogenesis in Rats," Cancer Res. 43:2477-2484 (1983).

18. Rogers, A. E., G. Lenhart, and G. Morrison. "Influence of Dietry Lipotropes and Lipid Content on Aflatoxin B_1, N-2-Fluorenylacetamide and 1,2-Dimethylhydrazine Carcinogenesis in Rats", Cancer Res. 40:2802-2807 (1980).

19. Norred, W. P. and A. E. Wade. "Dietary Fatty Acid-Induced Altertion of Hepatic Microsomal Drug Metabolism," Biochem. Pharm. 21:2887-2892 (1977).

20. Newberne, P. M., J. Weigert and N. Kula. "Effects of Dietary Fat on Hepatic Mixed Function Oxidases and Hepatocellular Carcinoma Induced by Aflatoxin B_1 in Rats," Cancer Res. 39:3986-3991 (1979).

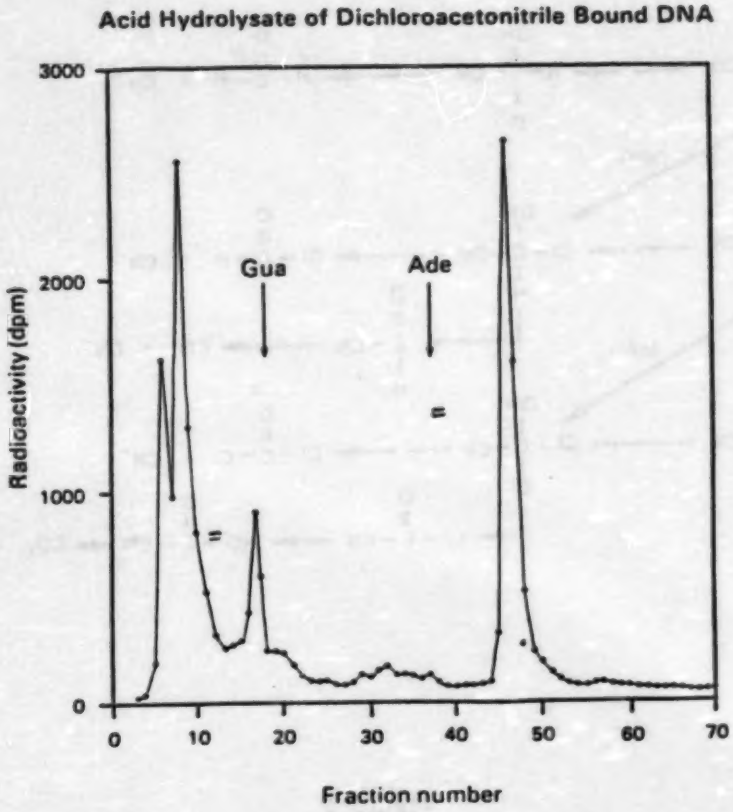
Acid Hydrolysate of Dichloroacetonitrile Bound DNA
3000
2000
1000
0
Radioactivity (dpm)
Gua
Ade
0
10
20
30
40
50
60
70
Fraction number

Figure 1

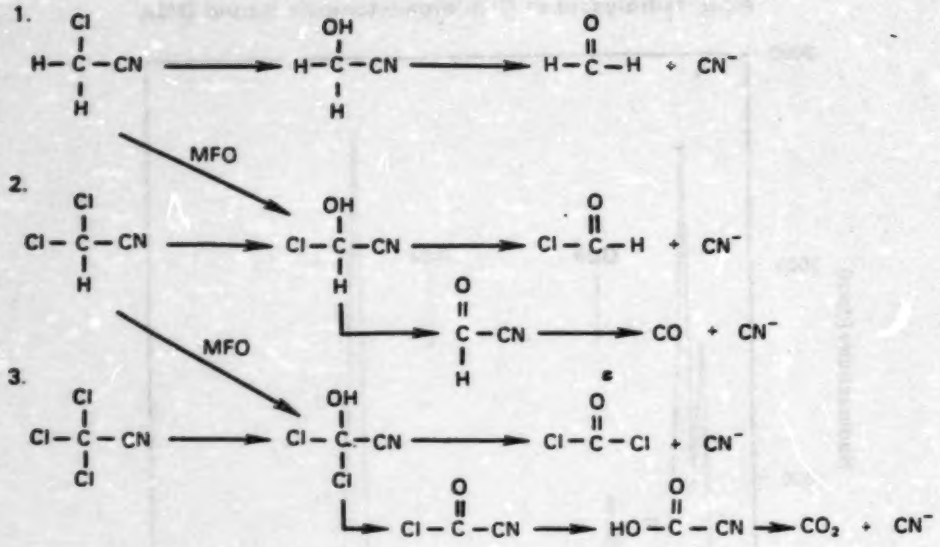

Figure 2